



FURTHER OBSERVATIONS ON VAGINAL MONILIAS AND VAGINAL MONILIASES.

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WHEN microscopic examination is made of stained smear preparations of vaginal discharge, large round or oval bodies usually described as yeasts or yeast-like cells are frequently observed. These are present in addition to an extensive bacterial flora which is always observed. As a rule the presence of these yeast-like bodies is straightway ignored on the presumption that they are of no clinical significance. Whether this presumption is justified or not yet remains to be proved. In the normal vaginal secretion they are present, if present at all, in extremely scanty numbers, being found neither in smears nor in cultures. On the other hand, they are often present in large numbers in cases of vaginitis associated either with an increased amount of vaginal secretion or with membrane formation.

Cultures of these organisms can readily be obtained by smearing a swab moistened with the vaginal discharge over the surface of a glucose-agar or a maltose-agar plate. By fishing and subculturing from the thick, creamy-white colonies that develop on the glucose-agar or the maltose-agar plates, pure cultures may be obtained without very great difficulty. Further study and investigation of the organisms so obtained shows them to belong to the genus *Monilia* Persoon 1797 of the class Fungi Imperfecti.

Under the name Fungi Imperfecti, Finckel gathered together all the forms of fungi, the complete history of which was unknown, and made this class in contradistinction to his other class of Fungi Perfecti, so that there is some ground for the naive remark of Lafar in his "Technical Mycology" that the Fungi Imperfecti are so called, not because the fungi in this group are themselves imperfect, but because our knowledge of them is imperfect.

They may be defined as follows: Fungi, almost invariably minute, but much larger than bacteria, in which asexual reproduction takes place by means of conidia produced on conidiophores which are either enclosed in perithecia, placed on discs, or unprotected.

The fungi thus defined were divided into two groups by Vuillemin, viz., the DEUTEROMYCETES and the HYPHALES. It is to the subclass Hyphales that the monilias belong, and the Hyphale of Vuillemin may be defined as Fungi Imperfecti with the hyphae more or less developed, less or more or less compact, superficial or subsuperficial or more rarely as in man, vertebrates and insects, endoparasite conidiophores never situate in closed or open receptacles. Reproduction by means of spores isolated

or in groups, situate on isolated or fasciculated hyphae. The monilias are included in the family Oosporaceae in the subclass Hyphales; and the relationship to the other genera of this family—the *Oospora* and the *Oidium*—is seen in the following classification:—

- A. Hyphae thin, short, simple, or nearly simple, terminating in chains of spores—*Oospora*, sugars not fermented with gas production.
- B. Hyphae not thin, often long and branched.
 - I. Sporophores simple and subsimple, typically with disjunction apparatus. Glucose completely fermented, gas being produced. Numerous budding forms in culture—*Monilia*.
 - II. Sporophores simple, septate, often with disjunction apparatus. Glucose not completely fermented, gas not being produced. Budding forms rare in culture—*Oidium*.

According to this classification the botanical definition of the monilias is as follows:—

Sporophores simple or subsimple, producing by constriction at their extremities a chain of large lemon-shaped conidia, often provided with a disjunction apparatus.

The usual definition, however, is as follows: Oosporaceae, possessing *in situ* budding forms and mycelial threads, which later are often long and branched; in cultures mostly budding forms but sometimes filaments in which thallospores of the blastophore type are found. Glucose and often other carbohydrate media are fermented with the production of gas.

Very closely allied to, and at present often included in this genus are the *Zymonema* of de Buermann and Gougerot, 1909, including the *Zymonema gilchristi*, more commonly called *Cryptococcus gilchristi*, which causes American blastomycosis, the *Parasaccharomyces* of de Buermann and Gougerot, 1909, and the *Parendomyces* of Querat and Laroche, 1909.

The genus *Monilia* is widely distributed in nature, a number of species having been found growing on dead leaves, fruits and decomposing wood. That a number of species may affect man was first pointed out by Castellani, who has shown their importance as the aetiological agents of thrush, bronchomycosis (*pro parte*), and some dermatomycoses; whilst Ashford attributes the causation of sprue to a monilia—*Monilia psilosis*, which is probably merely a synonym of *Monilia enterica* Castellani.

The classification of the monilias has been the subject of much confusion. Morphological grounds do not suffice for a classification, but by means of their biochemical reactions a fairly satisfactory classification has now been reached. By means of the sugar reactions it is possible to divide the species of *Monilia* into groups: (1) by the use of the following carbohydrates: glucose, levulose, maltose, galactose, saccharose, lactose, inulin.

1. *Balkanica* group.—Gas produced in glucose only. *Monilia balkanica* Castellani. *Monilia parabalkanica* Castellani.
2. *Krusei* group.—Gas produced in glucose and levulose. *Monilia krusei*, Castellani. *Monilia parakrusei*, Castellani.
3. *Pinoyi* group.—Gas produced in glucose, levulose, maltose. *Monilia pinoyi* Castellani. *Monilia nabarroii* Castellani.
4. *Metalondinensis* group.—Gas produced in glucose, levulose, maltose and galactose. *Monilia metalondinensis* Castellani. *Monilia pseudometalondinensis* Castellani.
5. *Tropicalis* group.—Gas produced in glucose, levulose, maltose, galactose and saccharose. *Monilia tropicalis* Castellani. *Monilia par-tropicalis* Castellani. *Monilia pulmonalis* Castellani. *Monilia nivea* Castellani. *Monilia insolita* Castellani. *Monilia enterica* Castellani.
6. *Guilthermondi* group.—Gas produced in glucose, levulose, saccharose and raffinose. *Monilia guilthermondi* Castellani. *Monilia pseudo-guilthermondi*.
7. *Macedoniensis* group.—Gas produced in glucose, levulose, galactose, saccharose and inulin. *Monilia macedoniensis*, *Monilia macedoniensoides*.
8. *Pseudotropicalis* group.—Gas produced in lactose and other carbohydrates. *Monilia pseudotropicalis*.¹
9. *Pseudolondinensis* group.—Gas produced in dextrin in addition to other carbohydrates. *Monilia pseudolondinensis* Castellani. *Monilia pseudolondinoides*, Castellani. *Monilia africana*, Macfie.
10. *Zeylanica* group.—No gas produced in any sugar.

In the vaginal discharge of cases of vaginitis, monilia belonging to groups *balcanica*, *pinoyi*, *metalondinensis* and *tropicalis* have been found in certain parts of the tropics, in the Balkans, in Italy and in England. More extended investigations will probably reveal other members of the genus in this condition. The members of Group 1 found were *Monilia balcanica* and *Monilia parabalcanica*; of Group 3, *Monilia pinoyi* and *Monilia nabarroii*; of Group 4, *Monilia metalondinensis*; and of Group 5, *tropicalis*. The principal characters of these organisms, together with other monilias, are set forth in the accompanying table.

In searching the literature we find several references to this subject; thus: Littauer described an aphthous vulvitis due to *Oidium albicans*. He believed that this condition begins as a primary vaginitis and is rarely found in children, but is common in diabetes and in the aged. He also mentions the cases of Plant in Hamburg, in which vaginal thrush and vaginal pruritis were associated with fungi of similar nature.

¹ It is important to note that many monilias after a few transplantations lose some of their fermentative characters or these are altered. Hence the determination of species is possible only when using recently isolated strains.

TABLE I.—SHOWING CHARACTERS OF VARIOUS MONILIAS.

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Von Herff has also recorded the occurrence of monilias in the vagina in cases of vaginitis and of vaginal pruritus, considering, however, that they were all comprised in the species *albicans* and *candida*. Loeser found *saccharomyces* among the microbic flora of the normal vagina and in cases with cervical catarrh with the formation of erosions, whereas Frank considers that in a perfectly healthy vagina only vaginal bacilli and *comma variabile* are found in the spread, and as the vagina approaches the pathological, more and more of the bacteria appear. Cases of vaginal thrush have also been described by Guilini, Hausmann, Vleemann, Mettenheimer and Panfilowicz.

SYMPTOMATOLOGY.

Two clinical types of vaginitis associated with monilias may, in our experience, be distinguished; in the first or membranous type, thrush-like patches of membrane are present on the mucosa (the so-called vaginal thrush); in the second or purulent type no such patches are present, but the secretion is thick and purulent. Where laboratory facilities do not exist cases of the purulent type of vaginal monilias are not rarely mistaken for gonorrhœa. The following illustrative case, which occurred in Ceylon, may be quoted: The surgeon in charge of the Colombo Lying-in Home had a case in which a pelvic operation was urgent; a thick purulent discharge was, however, noted, and active gonorrhœa was suspected, and he felt inclined to postpone the operation. A specimen of the discharge was sent to one of us for examination. No gonococci were found, but an enormous number of Gram-positive mycelial and conidial elements were present. Further investigation revealed the fungus to be *Monilia pinoyi*, Castellani.

Attention may also be directed to the presence of very numerous monilias in certain cases of chronic vaginal pruritus associated with muco-purulent discharge, although we cannot say whether the monilias really play a rôle in the causation of the pruritus. In these cases abundant irrigations of iodine (tinct. iodi 3j, aq. ad Oij) have answered exceedingly well, especially after a preliminary short course of hot bicarbonate of soda irrigations. We might also call attention to a variety of pruritus vulvæ and pruritus ani described by one of us (C.), caused by localization of fungi of the genus *Epidermophyton* in the ano-perineal region and on the external surface of the labia majora, without any objective symptom pointing to an epidermophytic or trichophytic infection. In such cases the fungus remains dormant, and apart from the pruritus, give rise to no symptoms, although on close examination not rarely a few red patches may be seen. The condition may be compared to the pruritus interdigitalis pedium caused by the localization to the skin of the toes of the same epidermophyton. Fungi in that localization, too, remain dormant, causing pruritus but no objective sign pointing to an epidermophytic condition.

Recently we have come across a most interesting case of extremely severe pruritus ani, pruritus vulvæ

and pruritus vaginæ in an elderly spinster, of some years' duration, which had been treated by different medical men in various ways without any distinct amelioration resulting. In the perianal region, on the perineum and on the labia majora a very few small reddish patches were seen, which it was thought might be due to a secondary pyogenic infection produced by scratching. There was an abundant purulent vaginal discharge and a very similar discharge from the anus, with symptoms of proctitis. A complete mycological and bacterial investigation was carried out, with the following results:—

1. From the scrapings of the perianal region and from the labia majora a fungus was grown with the characters of *Epidermophyton cruris* Castellani, 1905, the fungus which is the commonest cause of dhobie itch and pruritus interdigitalis pedium.

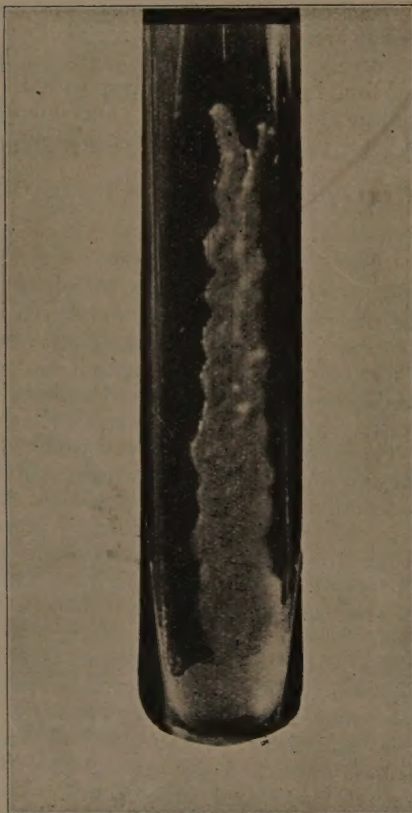
2. In the vaginal and in the rectal discharge enormous numbers of monilias were present, with very few bacilli; cultivations showed the fungus to be a strain of *Monilia pinoyi* Castellani.

The following treatment was given: creosote was administered by the mouth. Tinct. iodi, which in this case did not give rise to any local pain or irritation, was freely applied once a day to the anal-perineal region, the perineum and the external surface of the labia majora. For the first three days hot alkaline irrigations (two ounces of bicarbonate of soda to two pints of hot water) were given; afterwards, twice a day with iodine irrigation (tinct. iodi 3j, aq. ad Oij). Within two weeks the lady was completely free from the ano-perineal and vulvo-vaginal discharge, and the purulent rectal discharge had ceased entirely, whilst the vaginal discharge had decreased but had not quite disappeared. The monilias present had diminished enormously in number, but had not disappeared completely.

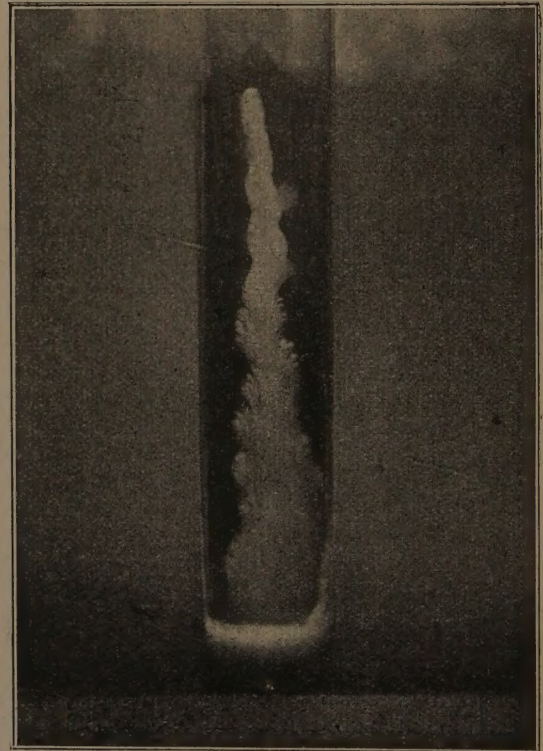
MONILIAS IN THE IDENTIFICATION OF CERTAIN CARBON COMPOUNDS.

Detection of Lactosuria.

Certain species of monilia, some of which were isolated from the vagina, have been employed by us in association with other micro-organisms as reagents in a new method of mycological analysis of carbohydrates and other compounds of carbon, theoretically devised by one of us (C.) in Ceylon some years ago. The differentiation, for example, of the various Fehling-reducing substances present in urine is readily accomplished by the application of this mycological method. By its use we have been able to recognize the following carbohydrates in urine, viz., glucose, lactose, galactose, saccharose, maltose, pentose, glycerol and inositol. It forms a ready and convenient method for recognizing the presence of lactose in the urine of pregnant and puerperal women, and for differentiating this substance from glucose and other Fehling-reducing substances. The technique of the method may be found in papers published by us in the *British Medical Journal*, 1917, vol. xvi, No. 5. We will limit ourselves to describing very briefly the detection of lactosuria.

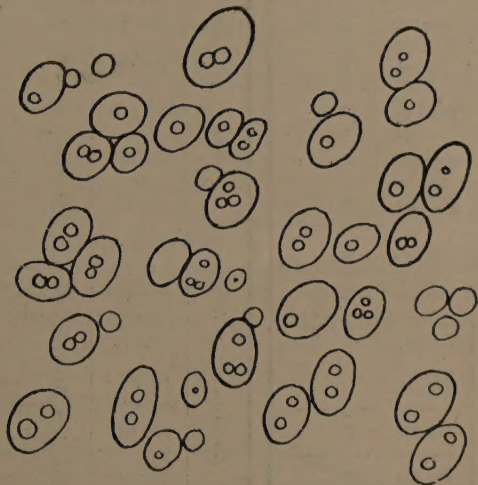


TUBE 1.—*Monilia tropicalis* Castellani.



TUBE 2.—*Monilia pinoyi* Castellani.

Lactose, as is well known, reduces Fehling's solution, and not infrequently lactosuria has been mistaken for true glycosuria. The detection of lactose by purely chemical methods is difficult for an ordinary medical man. In most textbooks it is stated that if a urine reduces Fehling and is not fermented by ordinary baker's yeast (so-called German yeast), the presumption is that the reducing substance is *lactose*. There are, however, two important sources of error. First, in our experience, quite a number of specimens of baker's yeast ferment lactose in addition to glucose, maltose, and other sugars; second, even if the baker's yeast should not ferment lactose this is not the only Fehling-reducing substance which may not be fermented by baker's yeast—the pentoses, for instance. If a urine which reduces Fehling is not fermented by ordinary yeast we cannot therefore come to the conclusion that it is a case of lactosuria. Our mycological or myco-bacterial method, on the other hand, renders possible the detection of lactosuria in a very easy manner.



The urine is collected aseptically, or if this is not feasible, is distributed into sterile tubes (each containing a small fermentation tube) as soon as passed, and then sterilized in Koch's stove for thirty minutes on two or three consecutive days, or the urine may be filtered through a sterile Berkefeld filter. It should never be autoclaved, as this procedure not rarely alters the chemical structure of the sugars. Two or three tubes of the aseptic urine to which one-third or the same amount of sterile sugar-free peptone water has been added, are inoculated with certain monilias and other micro-organisms as follows:—

(a) No. 1 tube is inoculated with *Monilia pseudotropicalis*. No. 2 is inoculated with *Monilia tropicalis*. The two tubes are placed in the incubator at 37° C. for three days and then the results are read. If No. 1 tube (*Monilia pseudotropicalis*) contains gas, and No. 2 (*Monilia tropicalis*) does not, we can come to the conclusion that in all probability the urine contains lactose. This is easily explained by

keeping in mind the biochemical reactions of *Monilia tropicalis* and *Monilia pseudotropicalis*. These two monilias differ only in regard to lactose and maltose, *Monilia pseudotropicalis* fermenting maltose but not lactose; a substance, therefore, which is fermented by *Monilia tropicalis* must in all probability be *lactose*. This is represented by the following mycological formula:—

<i>Monilia pseudotropicalis</i>	+	} = Lactose.
<i>Monilia tropicalis</i>	0	

(b) No. 1 tube is inoculated with *Bacillus neapolitanus* Emmerich. No. 2 tube is inoculated with *Bacillus pseudoasiaticus* Castellani. If after two days in the incubator at 37° C., No. 1 tube (*Bacillus neapolitanus*) contains gas and No. 2 tube does not contain gas, we can come to the conclusion that the urine contains lactose. The explanation lies in the fact that *Bacillus neapolitanus* and *Bacillus pseudoasiaticus* differ only in lactose as regards sugars and other carbohydrates found in pathological urines.

<i>B. neapolitanus</i>	+	} = Lactose.
<i>B. pseudoasiaticus</i>	0	

(c) Tube 1 is inoculated with *Bacillus coli*, tube No. 2 with *Monilia metalondinensis*. If after 48 hours there is fermentation in No. 1 tube and no gas in No. 2 tube, we can come to the conclusion that lactose was present, provided Fehling was positive.

Fehling	+	} = Lactose.
<i>Monilia metalondinensis</i>	0	
<i>B. coli</i> Escherich	+	

(d) In practice, the following procedure is very convenient. No. 1 tube is inoculated with *Bacillus coli* Escherich, No. 2 tube with *Bacillus paratyphosus* B Schottmüller. If fermentation occurs in tube No. 1 (*coli*) and does not occur in tube No. 2 (*paratyphosus*) we may come to the conclusion that the urine contained lactose, provided Fehling was positive. This is easily understood by remembering that *Bacillus coli* and *Bacillus paratyphosus*, besides lactose, differ in their action on raffinose and salicin, but raffinose and salicin are not Fehling-reducing.

Fehling	+	} = Lactose.
<i>B. coli</i> Escherich	+	
<i>B. paratyphosus</i> B Schottmüller	0	

Further details on the method may be found in our publication in the *Annales de l'Institut Pasteur* (November, 1922).

VAGINITIS ASSOCIATED WITH FUNGI OTHER THAN MONILIAS.

In addition to fungi of the genus *Monilia* Persoon, we may add that one of us (C.) has found occasionally in the vaginal secretion of native women suffering from very chronic vaginitis in the tropics, fungi of the genus *Oidium* (Link), *Aspergillus* (Micheli), *Sporotrichum* (Link), *Chladosporium* (Link), *Sterigmatocystis* (Cramer), *Cryptococcus* (Kützing, 1883), *Willia* (Hansen), *Vibrio-thrix* (Castellani), and others. Recently we have

rigid thickening of the wall. The protoconidium is transformed completely or partially into deuterconidia, but occasionally it elongates, forming a new conidiophore, or puts out branches which are capable of becoming conidiophores. As regards classification, there is only one genus, *Hemispora* Vuillemin, 1906, the definition of which is mycelial filaments, thin, hyaline, septated, ramified. Each conidiophore terminates into an ampulliform structure (protoconidium), which later divides into several spore-like segments (deuterconidia). Three species are known: *Hemispora stellata* Vuillemin, *Hemispora rugosa* Castellani, *Hemispora pararugosa* Castellani, Douglas and Thompson. The latter is probably only a variety of *H. rugosa*.

249 *Hemispora rugosa* Castellani, 1910 (*Syn. Monilia rugosa* Castellani, 1910.)

Definition.—*Hemispora* growing on all ordinary media, with a crinkled surface without asteroid colonies.

Remarks.—The growth on glucose agar is abundant, crinkled, or at times somewhat cerebriform; and in colour is amber, yellow, or brownish. Grows well on gelatine, which it liquefies very slowly, so much so that at first it was believed to be a non-liquefier. Gram-positive. Milk is not changed as a rule, but occasionally it undergoes a certain degree of peptonization, with a very small coagulum at the bottom of the tube. For sugar reactions see the table.

Hemispora pararugosa Castellani, Douglas and Thompson differs from *H. rugosa* in being a rapid liquefier of gelatine. *Hemispora stellata* Vuillemin is characterized by its asteroid colonies, and moreover, after a time glucose agar cultures develop a black pigmentation.

CONCLUSIONS.

1. In the normal vagina monilias and other fungi appear to be constantly absent.
2. In certain cases of vaginitis, vulvo-vaginitis, and of vulval vaginal and ani-perineal pruritus, monilias are abundantly present and may perhaps play some rôle in the ætiology of such cases, or at least produce the associated purulent or mucopurulent discharge.
3. Two principal types of vaginitis are associated with the presence of monilias, namely (a) the membranous type, presenting white patches of membrane on the vaginal mucosa, the so-called "vaginal thrush," and (b) the purulent type, with thick

purulent vaginal discharge containing enormous numbers of monilias and sometimes simulating gonorrhœal infection.

4. Monilias, along with other micro-organisms, are employed in the authors' mycological method of identification of carbohydrates and other carbon compounds. This method is especially useful in the detection of lactosuria.

5. Attention is also directed to the presence of several genera of fungi other than the monilias in the vaginal secretion of cases of chronic vaginitis. A case of vaginal aspergillomycosis and a case of vulvar hemisporosis have been briefly described.

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